



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY
OF *ARTOCARPUS ALTILIS* (MORACEAE)
AND *MICROMELUM MINUTUM* (RUTACEAE)**

SHIREEN SHAHARINA BT MOHAMED SHAMAUN

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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

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By

SHIREEN SHAHARINA BT MOHAMED SHAMAUN

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in fulfilment of the Requirements for the Degree of Master of Science**

August 2008



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of requirements for the degree of Master of Science

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Chairman : Professor Mawardi Rahmani, PhD

Faculty : Science

The species of *Artocarpus altilis* from the Moraceae family and *Micromelum minutum* from the Rutaceae family were phytochemically studied and screened for their biological activities. The first species, *Artocarpus altilis* (Parkinson) Fosberg was collected from Kelantan and Pahang. *Micromelum Minutum* (G. Forst.) Wight & Arn. was collected from Sepilok, Sabah. The crude extracts and isolated compounds were screened for antimicrobial, cytotoxic and antioxidant activities using disc diffusion method, 1, 2-Diphenyl-2-picrylhydrazyl (DPPH) and Microculture Tetrazolium Salt (MTT) assays respectively. The microbes used for antimicrobial tests were methicilin resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Detail study on the bark of *Artocarpus altilis* afforded cycloartocarpin (51), artocarpin (52), morusin (53), cycloartobiloxanthone (54) and artoindonesianin V (55). Chemical investigation on the root of *Artocarpus altilis* have resulted in the isolation of two new flavonoids, hydroxyartocarpin (56) and artocarpinin (57). Urs-12-en-3 β -ol (58) and 6-methyl- β -D-glucopyranose (60) were isolated from the leaves of *Micromelum minutum* along with a coumarin known as 8-hydroxyisocapnolactone-2', 3'-diol (59).

The leaves crude extract of *Micromelum minutum* gave strong cytotoxic activity towards HL 60 cell line and exhibited free radical scavenging activity towards DPPH. However, no activity was observed in the crude extract of *Artocarpus altilis*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA
ARTOCARPUS ALTILIS (MORACEAE) DAN
MICROMELUM MINUTUM (RUTACEAE)**

Oleh

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Species *Artocarpus altilis* daripada famili Moraceae dan *Micromelum minutum* daripada famili Rutaceae telah dikaji secara fitokimia dan diuji aktiviti biologi mereka. Spesies–spesies ini adalah *Artocarpus altilis* (Parkinson) Fosberg yang telah diperolehi daripada Kelantan dan Pahang bagi setiap bahagian pada spesies ini dan *Micromelum Minutum* (G. Forst.) Wight & Arn. yang diperolehi daripada Sepilok, Sabah.

Ekstrak mentah telah diuji aktiviti antimikrob, antioksidan dan sitotoksik dengan menggunakan kaedah peresapan cakera, 1, 2-Diphenyl-2-picrylhydrazyl (DPPH) dan garam Mikrokultur Tetrazolium (MTT). Mikrob yang telah digunakan untuk ujian

antimikrob ialah *Staphylococcus aureus* yang resistan kepada methicilin (MRSA), *Bacillus subtilis* dan *Pseudomonas aeruginosa*.

Kajian terperinci ke atas kulit batang *Artocarpus altilis* telah menghasilkan sikloartokarpin (51), artokarpin (52), morusin (53), sikloartobiloxanthone (54) dan artoindonesianin V (55). Kajian kimia ke atas akar *Artocarpus altilis* telah menemui dua sebatian flavonoid yang baru, hidroksiartokarpin (56) disamping artokarpinin (57). Urs-12-en-3 β -ol (58) dan 6-metil- β -D-glukopiranos (60) telah ditemui daripada daun *Micromelum minutum* bersama dengan sebatian kelas koumarin yang dikenali sebagai 8-hidroksiisokapnolakton-2', 3'-diol (59).

Ekstrak daun bagi *Micromelum minutum* menunjukkan kesan aktiviti sitotoksik yang kuat terhadap sel HL 60 dan mempunyai aktiviti radikal bebas terhadap DPPH.

Walaupun demikian, tiada aktiviti yang diperolehi daripada ekstrak *Artocarpus altilis*.

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I certify that an Examination Committee met on 13th November 2008 to conduct the final examination of Shireen Shaharina Mohamed Shamaun on her Master of Science thesis entitled “Chemical Constituents and Biological Activity of *Artocarpus altilis* (Moraceae) and *Micromelum minutum* (Rutaceae)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the degree of Master of Science.

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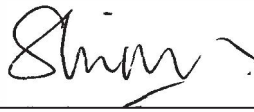
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DECLARATION

I declare that this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



SHIREEN SHAHARINA BT MOHAMED SHAMAUN

Date: 24th November 2008

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LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	delta (chemical shift in ppm)
λ_{\max}	maximum wavelength in nm
μg	microgram
μL	microliter
<i>br</i>	broad
^{13}C	Carbon-13
$^{\circ}\text{C}$	degree in Celsius
CC	column chromatography
CDCl_3	deuterated chloroform
CHCl_3	chloroform
cm^{-1}	per centimeter
COSY	Correlated Spectroscopy
<i>d</i>	doublet
<i>dd</i>	doublet of doublets
<i>ddd</i>	doublet of doublets of doublets
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	dimethyl sulphoxide
EtOAc	ethyl acetate
EIMS	Electron Impact Mass Spectrometry

g	gram
GC-MS	Gas chromatography-Mass Spectroscopy
^1H	proton
hex	hexane
Id	Inhibition diameter
<i>t</i>	triplet
<i>s</i>	singlet
<i>m</i>	multiplet
MeOH	methanol
m.p.	melting point
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
Pet-ether	petroleum ether
TLC	Thin Layer Chromatography
IR	Infrared
UV-Vis	Ultraviolet-Visible
ϵ	molar absorptivity



CHAPTER I

INTRODUCTION

GENERAL INTRODUCTION

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutically active compounds, natural products still remain as one of the best reservoirs of new structural types (Kurt Hostettmann, 1991). Over 120 pharmaceutical products currently in use are plant derived and 75% of these were discovered from plants used in traditional medicine.

After centuries of empirical use of herbal preparations, the isolation of active alkaloids such as morphine, strychnine, quinine etc in the early 19th century marked a new era in the use of medicinal plants and the beginning of modern medicinal plant research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period.

Medicinal plants are used basically in two different forms: (i) as complex mixtures containing a broad range of constituents (infusions, essential oils, tinctures, extracts); and (ii) as pure, chemically defined active principles. Pure compounds are generally employed when the active principles of a medicinal plant exhibited strong, specific activity or have a small therapeutic index, requiring accurate and reproducible dosage.

On the other hand, the use of extracts, tinctures etc is appropriate for plants exhibiting weaker or less specific pharmacological activities and if the principles of a medicinal plant are as yet unknown.

The process that leads from the plant to a pharmacologically active, pure constituent is very long and tedious, and requires a multidisciplinary collaboration of botanists, pharmacologists, chemists, pharmacognosists and toxicologists (M. Hamburger *et al.*, 1991).

The Family of Moraceae

Amongst the various tropical plants that has shown interesting chemistry and biological activity is the *Artocarpus* species of Moraceae family. This genus of plant is frequently found in lowland forests especially at villages in Malaysia. The family comprises of about 40 genera and over 1000 species of plants widespread in tropical and subtropical regions, less common in temperate climates. The popular plants of this family are fig, jackfruit, mulberry and Osage-orange.

Normally the member of the genus can grow up to 30 meters in height and some species produced sweet and succulent flesh. At least 30 species of this genus are available in our tropical forest and only a fraction has been investigated. The potential of discovering interesting compounds with good biological activity will be there for further exploitation.

The Species of *Artocarpus altilis*

The generally accepted name for breadfruit is *A. altilis* (Parkinson) Fosberg, which has taxonomic priority and replaced *A. incisus* (Thunb.) 1781 and *A. communis* Forst. 1776. These names for breadfruit are based solely on specimens or descriptions of seedless Tahitian breadfruit collected during Captain Cook's voyages in 1768-1771.

Locally known as 'sukon' or breadfruit, *Artocarpus altilis* is a multipurpose tree crop that is primarily used for its nutritious, starchy fruit. It is the main staple crop in many areas of the Pacific and supplements other staple foods for home consumption elsewhere. It generally has little commercial use but is becoming an export crop of the Caribbean and Pacific Islanders. Breadfruit originated in the western Pacific, with New Guinea and associated islands such as the Bismarck Archipelago being the centre of diversity for wild seeded forms of *Artocarpus altilis* (Parkinson) Fosberg. Few-seeded and seedless forms occur throughout the Pacific Islands with the greatest diversity found in the eastern Pacific in Polynesia. Seedless breadfruit has been widely distributed throughout the tropical world (Ragone, 1997).

The latex, leaves, roots and bark of the breadfruit are used medically in the Pacific Islands. Latex is commonly used to treat skin ailments and fungal diseases such as "thrush", which is also treated with crushed leaves. Diarrhea, stomachaches and dysentery are treated by internally taking the diluted latex. The root is used as an astringent and as a purgative, while the macerated root is used to treat skin ailments. Throughout many islands in the Pacific the bark is used to treat headaches. The yellowing leaf is brewed into tea and taken to reduce high blood pressure in the West

Indies (Ragone, 1997). It is thought that the tea also controls diabetes. A complex organic acid in the leaf extract (gamma-aminobutyric acid) is the active ingredient, but it is not clear whether the extract is effective in lowering blood sugar or whether the test for sugar is masked in the presence of the extract. Research on the efficacy of breadfruit extracts from various parts of the plants has shown promising results. The leaves are used to treat liver disease and fevers in Taiwan (Lin *et al.*, 1992), and an extract from the flowers was effective in treating ear edema (Koshihara *et al.*, 1988). Bark extracts exhibited strong cytotoxic activities against leukemia cells in tissue culture.

The Family of Rutaceae

Family of the flowering plants belonging to the order of Sapindales are valuable source of edible fruits and as ornamentals. Known as the citrus family, the Rutaceae includes woody shrubs, trees and a few herbaceous perennials which consist of 160 genera and 1,700 species distributed throughout the world, especially in warm temperate and tropical regions.

The Species of *Micromelum minutum*

Micromelum minutum are commonly known as *Chemama*, *Chememar*, *Chemama jantan*, *Cherek-cherek*, *Cherek puteh*, *Timah-timah* (in common with *Ilex*), *Titimah*, *Titimah betina*, *Kematu* (for kemantu is an example connected with ghosts, which is a name of *Clausena*), *Mali-mali* (in common with *Leea*), *Kayu saga*, *Kerman* (in common with *Bridelia*), *Jejawi*, *Beringin* (last two names in common with *Ficus benjamina*) and *Lankok* (Flora Mal. Penins., 1922). This is small tree found from Indo-China to the

Pacific; in the Peninsula it occurs in the hilly parts of the southern half and in the north-west.

The samples of *Micromelum minutum* for this research were collected from Sabah and investigated for their chemical constituents and biological activities. *Micromelum minutum* are widely used in several traditional medicines and the plant is known to contain coumarins, some are active compounds which showed good cytotoxic activities. In the north-eastern Sumatra, it is used in the treatment of fever. As in southern Sumatra, the bitter roots are chewed with betel for coughs. In Malaya, the poultice of boiled roots is applied for ague. The timber is light but said to be durable and used for houses (Burkhill, 1935).

The objectives of studying the chemical constituents and biological activities of *Artocarpus altilis* (root and bark) and *Micromelum minutum* (leaves) are mainly to:

1. extract each samples using different solvents
2. isolate and purify the compounds using chromatographic methods
3. characterize the pure compounds using modern spectroscopic methods
4. screen the biological activities of the extracts for antioxidant, cytotoxic and antimicrobial activities.